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BOTANICAL GAZETTE

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SPERMATOGENESIS, OOGENESIS, AND FERTILIZATION IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 107

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(WITH PLATES VI-VIII)

Historical review

The pteridophytes have been the source of important contributions to our knowledge of sperms in the plant kingdom, and investigations upon spermatogenesis in pteridophytes are already numerous. Historically, however, the motile sperm which first attracted attention was that of the algae, and among them especially the sperm of *Chara*.

From the early studies of spermatogenesis in *Chara* it was generally believed by NÄGELI (69), METTENIUS (62), HOFMEISTER (38), STRASBURGER (83), and SACHS (74) that the nucleus, before the formation of the sperm, dissolves into cytoplasm to form a homogeneous slimy protoplast from which the body of the sperm is organized. HOFMEISTER, STRASBURGER, and SACHS held a similar view concerning spermatogenesis in some forms of bryophytes and pteridophytes.

The first definite statement that the nucleus does not dissolve before the formation of the sperm was made by SCHMITZ (77) from a study of *Chara* and a number of mosses. He writes that the body of the sperm is produced by a direct change of form of the nucleus, whose peripheral layer becomes thickened to form a spirally coiled band, while the inner part becomes loose and forms a vesicle. The surface layer of the anterior end of the sperm, bearing cilia, consists of the cytoplasm which envelops that part of the nucleus.

Most of the papers concerning spermatogenesis in pteridophytes were published after the appearance of SCHMITZ's study of *Chara*, by such investigators as CARNOY (16, in *Hymenophyllum*), BELAJEFF (1, in *Isoetes*; 2, 4, in *Gymnogramme* and *Equisetum*), ZACHARIAS (102, in *Pteris*), CAMPBELL (12, in *Onoclea*, *Asplenium*, *Gymnogramme*, *Adiantum*, *Alsophila*, and *Ceratopteris*; 13, in *Pilularia*; 14, in *Osmunda*), BUCHTIEN (10, in *Equisetum*), LECLERC DU SABLON (61, in *Cheilanthes*), GUIGNARD (31, 32, in *Angiopteris*, *Pilularia*, and *Equisetum*), SCHOTTLANDER (78, in *Gymnogramme*), and STRASBURGER (85, in *Marsilia*).

These studies begin with the structure of spermatids and are chiefly devoted to details of the events which occur within the spermatids during their transformation into sperms. Since the forms investigated were different, it is natural that the results should diverge in some particulars; but so far as the constituents of the mature sperm are concerned, the views fall into two categories. One group of authors, as ZACHARIAS, LECLERC DU SABLON, and BELAJEFF, believes that the spiral body of the sperm consists of a nucleus always enveloped by a layer of cytoplasm, which becomes the main constituent of the anterior coiled part of the body where cilia are produced; nuclear material does not extend to the anterior part. The other authors mentioned above hold that the nucleus is the main constituent of the body of the sperm, its anterior coiled part being enveloped with cytoplasm on which cilia are borne. Most observers of both groups agree that the cilia are produced from the cytoplasm, and that the vesicle (*Blase*) which is attached at the posterior end of the sperm is cytoplasmic in nature.

Most of the investigation of the origin and nature of the cilia-bearing organs of the sperms of pteridophytes has been done since the discovery of motile sperms and cilia-bearing structure in gymnosperms was made by IKENO (40, 41, 42, 43), HIRASÉ (36, 37), and WEBBER (94, 95). The first paper that dealt with the details of the origin of cilia-bearing structures in the sperms of pteridophytes was that of BELAJEFF. Following his three preliminary notes (3, 4, 5), he published in 1898 an account (6) of spermatogenesis in *Gymnogramme* and *Equisetum*. Two deeply staining bodies (which he called *Nebenkern*) appeared on opposite sides of the nucleus previous

to the final mitosis in the antheridium which differentiates the spermatids. At this final division each spermatid receives a blepharoplast which lies close beside the nucleus. The blepharoplast begins to elongate and its elongation is followed by that of the nucleus, so that both structures form two parallel bands which take a spiral form.

SHAW's paper (80) on *Onoclea* and *Marsilia* appeared almost simultaneously with the foregoing contribution of BELAJEFF. He investigated the cell divisions preceding the formation of the spermatid in *Marsilia* and found two small bodies, which he called "blepharoplastoids," first in the daughter nuclei after the mitosis which differentiates the spermatid grandmother cell. There is one blepharoplastoid, therefore, for each nucleus of the grandmother cell of the spermatid. This blepharoplastoid divides, but the halves remain close together and pass to one side of the cell. With the next mitosis which occurs in the grandmother cell two new blepharoplasts appear at the pole of the spindle, and they accompany each daughter nucleus after this mitosis into the spermatid mother cell. Then each divides, and after the division of the spermatid mother cell, a blepharoplast is included in each spermatid.

The next year BELAJEFF (7) studied *Gymnogramme* and also the same species of *Marsilia* which SHAW had investigated. He found centrosome-like bodies at the poles of spindles in various mitoses preceding the formation of the spermatids with their unquestioned blepharoplasts, and he also found the blepharoplasts at the poles of the spindle. From this he drew the conclusion that the blepharoplast of *Marsilia* holds the same relation to the pole of the spindle as does a centrosome.

THOM (88) in the same year observed a blepharoplast in the spermatids of *Adiantum* and *Aspidium* as a round body beside the nucleus. He states that the disappearance of nucleoli at the time of the appearance of the blepharoplast would suggest a relationship between them. According to his observation the modification of the blepharoplast into a band structure is considerably delayed after the transformation of the nucleus.

No contribution based upon the original investigation of spermatogenesis in pteridophytes has been published since THOM's paper

appeared. However, the problem concerning the nature of the blepharoplast and its relation to the centrosome, not only in pteridophytes but in various groups of plants, has been discussed by STRASBURGER (87, with a critical review of the works on this subject), DANGEARD (21, on algae), TIMBERLAKE (89, on algae), WEBBER (96, on *Zamia*), IKENO (45, on *Marchantia*; 46), DAVIS (24), and some others.

Oogenesis in pteridophytes has not aroused as much interest as spermatogenesis. Oogenesis in Filicales has been observed in various forms by JANCZEWSKI (49), GOEBEL (29), BRITTON (9), CAMPBELL (15), and many others.

Morphological investigations of the processes of fertilization in pteridophytes have been published by STRASBURGER (83, on *Pteris* and *Ceratopteris*), HANSTEIN (33, on *Marsilia*), CAMPBELL (13, on *Pilularia*; 14, on *Osmunda*), SHAW (79, on *Onoclea*), and THOM (88, on *Adiantum* and *Aspidium*). A few points of cytological interest in their studies concerning fertilization in pteridophytes may be summarized as follows:

In his paper on *Osmunda*, CAMPBELL writes that in several instances, when the archegonium seemed about ready to open, an appearance was observed that looked very much like the formation of a true polar body, and was not to be compared with the ventral canal cell. He suggests that this structure, like the polar body, may be derived from the division of the nucleus of the egg, his reason for the suggestion being the apparent diminution of the nucleus of the mature egg at the time of the appearance of the structure, although actual division was not observed.

In *Onoclea* SHAW states that before the archegonium opens, the egg is depressed above, and the nucleus of the egg is flattened, due to the pressure caused by the swelling of the wall of the ventral canal cell. As soon as the archegonium opens and the disorganized contents of the neck canal cells are expelled, the egg becomes turgid. After the sperm has entered the egg at the receptive spot, the egg collapses, and he suggests that this prevents the penetration of more than one sperm. MOTTIER (67) expressed some doubt whether the collapsed appearance is really normal.

In *Pteris* STRASBURGER (83) observed the formation of a mem-

brane around the fertilized egg within a very few minutes after the entrance of the sperm, while SHAW in his paper (79) on *Onoclea* found no evidence that a membrane of appreciable thickness was formed immediately after the entrance of sperm.

In his account of *Adiantum* and *Aspidium*, THOM finds that when the sperm reaches the nuclear membrane, the nuclear part of the sperm finds or breaks an opening into the egg nucleus and cytoplasmic envelope, while the blepharoplast and the cilia are left out in the cytoplasm of the egg. Whether blepharoplast and cilia are left out in the cytoplasm SHAW leaves in doubt.

That the egg nucleus during the entire process of fertilization is in the resting condition and that the sperm enters the egg nucleus before undergoing any change in form or visible structure, are points of agreement among these authors in their studies on *Adiantum*, *Aspidium*, *Onoclea*, and *Osmunda*. One peculiar case, different from the foregoing results, was reported for *Pilularia* by CAMPBELL (13), who finds that a sperm nucleus assumes a loose and more granular structure and rounds up before entering to unite with the nucleus of the egg.

No work has been published showing details of the behavior of chromosomes during spermatogenesis, oogenesis, and fertilization in pteridophytes, nor has the organization of the figure of first segmentation division ever been followed.

The present investigation of this subject, with a study of sporogenesis which was considered in my recent paper (101), was carried on to trace out the complete history of the chromosomes throughout the life-cycle of *Nephrodium molle* Desv. The former paper, together with the present investigation, may serve as an introduction to the study of apogamy in *Nephrodium*, which will appear in a subsequent paper.

Material and methods

Gametogenesis and fertilization were studied from prothallia raised from spores in the greenhouse of the Hull Botanical Laboratory during the five months beginning October 1906. The spores for this culture were collected from leaves of the same individuals of *Nephrodium molle* that furnished material for the study of sporogenesis.

Various fluids were used for killing and fixing. The best fixation for the present studies was obtained in the material killed and fixed in

the following solution: 1 per cent. chromic acid 25°C, 1 per cent. glacial acetic acid 15°C, 1 per cent. osmic acid 10°C, water 50°C. The methods used after the material was thoroughly washed were similar to those given in the previous paper on sporogenesis (101).

Description

VEGETATIVE MITOSIS IN PROTHALLIA

Immediately after the germination of the spore, the nucleus in the prothallial cell is quite large, but shows a tendency to diminish gradually during the further development of the prothallia into many-celled stages. The events of the mitosis in any vegetative cell of a prothallium are not only repetitions of the same type, but they are similar to those of the sporophyte. For this reason no detailed account will be made at this time, but a few essential points deserve mention.

The chromatin reticulum of the resting nucleus of the prothallia, which has a ragged structure like that in a vegetative cell of the sporophyte, becomes transformed into a spirem that runs an irregular course within the nucleus, many tangled parts or loops coming into close contact with the membrane. The spirem then becomes rather smooth and uniform in thickness and very likely there is present a continuous spirem (*fig. 1*). A kinoplasmic cap of conical shape appears at each pole of the nucleus, consisting of a few fibers arranged along the lateral edges of the cone. With the dissolution of the nuclear membrane at the two poles, the fibers push in and attach themselves to the chromosomes which have just segmented from the spirem (*fig. 2*).

The points at which segmentation of the spirem into chromosomes may occur have a certain regularity in relation to location within the nucleus. Although it is impossible to give an accurate explanation of the mechanism at present, the fact that many free ends of the chromosomes, immediately after segmentation, are directed toward the poles may prove that the segmentation took place in bent points of loops. Longitudinal splitting of the chromosomes appeared after the completion of this segmentation.

Contemporary with the entire dissolution of the nuclear membrane, the chromosomes show a tendency to arrange themselves parallel to an axis passing between the two poles (*fig. 3*). The daughter halves

of chromosomes either remain in contact side by side throughout their length, or diverge in the middle part, remaining attached only at the ends; but the latter case is of short duration and soon they become reunited in pairs. The chromosomes now shorten and assume an L-shape, and there is established an early condition of an equatorial plate in which the vertical arms of the L-shaped chromosomes are parallel and directed toward the pole, while the horizontal arms lie in the equatorial plane; the spindle threads are attached at the ends directed toward the periphery of the equator (*fig. 4*). A polar view in this stage shows that the horizontal arms hold almost a radial arrangement, while the vertical arms are visible only in the optical section of their ends (*fig. 5*). The shortening and thickening of the chromosomes advance farther until they become short, rod-shaped, and arranged in the equatorial plate.

The separation of daughter chromosomes begins at once (*fig. 6*). As a rule, it takes place similarly in each chromosome, so that the daughter chromosomes proceed toward the pole as a set (*fig. 7*). When the set of daughter chromosomes has reached the pole, they remain for a short time as regularly arranged and straight chromosomes (*fig. 8*), the polar view of which clearly shows that the number of the chromosomes is 66 or 64 (*fig. 9*). The daughter chromosomes grouped at the pole become drawn together tightly and vacuolization begins. When the nuclear membrane is formed, the chromatin structure of the daughter nucleus shows polarity (*fig. 10*). The formation of the cell plate is like that described for the vegetative mitosis of the sporophyte.

SPERMATOGENESIS

A detailed description of the mitoses which take place from the cutting-off of an antheridium initial from a superficial cell until the formation of a spermatid mother cell will be omitted. The number of chromosomes is always 66 or 64. The only point which seems noteworthy is that in the central or primary spermatogenous cell there were observed in the majority of cases threadlike structures imbedded in the cytoplasm, sometimes very densely crowded together near the cell wall. The length of these threads was various, and they were a little more slender than the spirem that is usually present in the mitoses of spermatogenous cells. No such structure was ever observed

in the superficial cells or in the antheridium initial from which the central cell is derived, and consequently this structure may originate in the central cell. It does not seem to play any important rôle in the nuclear division. As successive mitoses proceed, it becomes more faintly stained, less conspicuous, and finally becomes impossible to differentiate by staining; but in rare cases it still remains, filling the whole cytoplasm up to the eight- or sixteen-cell stage of the spermatogenous cells. A structure of similar appearance was observed in the central cell of the archegonium (*figs. 41, 41a*).

The spermatid mother cell is characterized by the first appearance of blepharoplasts. In general, the spermatogenous cells, in spite of their small size compared with the vegetative cells of the prothallia, contain large nuclei. The cytoplasm seems generally destitute of plastids and has a very fine fibrillar structure, and the membrane is delicate. The mitoses which take place in the spermatogenous cells are conspicuous on account of the comparatively large amount of chromatin, but otherwise they resemble those of the vegetative cells.

In the telophase of the mitosis, previous to the formation of the spermatid mother cell, the groups of daughter chromosomes, having reached the poles, begin to be vacuolized. Detailed study of this stage shows that the formation of the membrane around the mass of vacuolized chromosomes, or young daughter nucleus, is a little delayed in the polar region, which appears concave from the side view (*fig. 11*). The nucleus when viewed from the pole shows that the chromatin material resulting from the vacuolized chromosomes is very scanty in the polar region and three or four nucleoli are always present (*figs. 12, 13*); such a structure of the nucleus may be regarded as a kind of polarity. If there could possibly be any migration of nucleoli from the interior of the nucleus to the cytoplasm, it would certainly occur in such a critical stage of the nucleus, but there was not observable any peculiar differentiation within the cytoplasm that might warrant the supposition.

As the young daughter nucleus grows in size and assumes a spherical form, the chromatin loses its peculiar arrangement and becomes transformed into the irregular ragged reticulum of the resting condition. Then there are observed by their differential staining two small bodies, the primordia of the blepharoplasts, lying within the

cytoplasm at the opposite sides of the nucleus. The first differentiation of the blepharoplasts by stains is very sudden, and they are unexpectedly large, quite surprising in fact, for one would imagine that they should appear at first as very small granules of hardly distinguishable size. The blepharoplasts move toward the nucleus, and during mitosis they hold positions near the poles of the spindle, without any noticeable increase in size (*figs. 14-19*). Sometimes (not often) it is observed that the blepharoplasts lie just at the poles of the spindle, but evidently this does not seem to mean that the blepharoplasts function as centrosomes; instead, the blepharoplasts in this case may have happened to be caught accidentally in the focal centers of the spindle.

In the anaphase of this mitosis 66 or 64 chromosomes are always counted (*fig. 20*). After the telophase each daughter cell of the spermatid mother cell, or each spermatid, contains a single blepharoplast, which now lies very close to the concave side of the nucleus.

When the cell plate is just being completed, a new body appears near the nucleus of the spermatid far away from the blepharoplast (*fig. 21*). For the sake of convenience, I called this body a *Nebenkern* in the preliminary note. It should be understood that this term is used simply as a temporary designation, which must be replaced by a more accurate one. The *Nebenkern* in *Nephrodium* always appears in the location previously occupied by the central spindle of the last mitosis. This spindle has almost disappeared when the *Nebenkern* appears. Accordingly, it is not probable that the *Nebenkern* originates from the spindle.

The spermatids grow to a certain stage and become almost free from one another, so that the antheridium now contains eight to thirty-two polyhedral or spherical spermatids, with very delicate membranes. During the growth of the nucleus of the spermatids the nuclear reticulum becomes transformed into a ragged network, very rich in irregular chromatin clumps, but with scanty branched strand structures. One or two nucleoli are always present (*fig. 22*). The blepharoplast, a round granule by this time, becomes applied to the nuclear membrane, and the *Nebenkern*, also a round body a great deal smaller than the blepharoplast, lies in various locations within the cytoplasm.

Now the modification of the blepharoplast begins. While the

spermatid does not grow further, the nucleus and the blepharoplast increase in size during this metamorphosis, and the space occupied by the cytoplasm consequently gradually diminishes. The blepharoplast, now round in shape, lies very close to the nucleus and elongates along its spherical surface (*figs. 23, 24*), so that the surface view of the blepharoplast at this time shows a rhomboidal outline (*fig. 23*) and its cross-section is crescent-shaped, the concave surface being in close contact with the nuclear membrane (*fig. 24*). The elongation of the blepharoplast now proceeds farther. *Fig. 26* shows the section of the blepharoplast at this stage, cut parallel with the axis of elongation. The cross-section of the blepharoplast is shown in *fig. 27*, and a part of the structure viewed from the surface is given in *fig. 25*.

Until this time the blepharoplast is alike at both ends, and no modification was ever observed in the structure of the nucleus; but soon after the blepharoplast has elongated into a semicircular band, a very complicated modification follows in both nucleus and blepharoplast. One end of the blepharoplast becomes wedge-shaped and loosely laid along the spherical surface of the nucleus (*fig. 28*), while the other end, which still remains pointed, comes into close contact with the nucleus and finally coalesces with it (*figs. 28, 29*). The elongating blepharoplast now takes a spiral direction and has a tendency to form a coiled ribbon-like structure.

While the foregoing modification is occurring in the blepharoplast, the nucleus undergoes a characteristic transformation of form. The distribution of the ragged chromatin clumps within the nucleus becomes irregular; at certain regions of the nuclear cavity they become grouped very densely; while in other parts the chromatin substance is scattered. The nuclear membrane, very delicate at this time, seems to be easily influenced by any change which occurs in the interior of the nucleus, so that the region where the chromatin clumps are densely crowded may protrude above the spherical surface of the nucleus, while the region with scanty chromatin material may form a depression or furrow. This unevenness in the form of the nucleus, brought about by the irregular aggregation of the ragged chromatin clumps in different regions of the interior, develops in such a direction that the nucleus becomes metamorphosed into a coiled structure.

When the nucleus has almost assumed the coiled form, one end of

the coil takes on a round sausage-shape (*fig. 30*), which gradually passes into a band structure (*fig. 29*). This band becomes more and more narrow, with a gradual diminution of its width, but this diminution is compensated for by a coalescence with the tapering end of the band-shaped blepharoplast; so that now the tapering end of the blepharoplast runs with the nuclear band as far as the latter structure goes, and finally the blepharoplast ends in the wedge-shaped band. Then the outer surface of that part of the coiled band, which is composed of the blepharoplast and nucleus by their parallel coalescence, becomes covered by the blepharoplast, which grows extensively.

The structure of the coiled nuclear region becomes more and more compact, during this metamorphosis of the nucleus, by the gradual condensation and aggregation of clumps of the chromatin reticulum. Thus the general structure of the coiled sperm is organized within the spermatid. By this time, numerous cilia are observed growing on the surface of the coiled band of the blepharoplast (*fig. 31*). During this modification of the nucleus and blepharoplast, the *Nebenkern* is always present in a definite position close to the nucleus, and when the coiled form of the sperm is established, the *Nebenkern* is observed near the blunt end of the sausage-shaped portion without entering into the construction of the body of the sperm (*fig. 32*).

The mature sperm when observed within the spermatid consists of 2.75 coils, 1.5 of which are composed of the blepharoplast with the nuclear substance lining a certain distance, and the rest exclusively of the nucleus. When the spermatids are discharged from the antheridium the anterior part of the sperm, which consists of the blepharoplast with cilia, first protrudes from the membrane of the spermatid, the membrane being so delicate at this time that it looks like a thin film of cytoplasm. As soon as the sperm begins to swim freely, the cytoplasmic substance with the *Nebenkern* is attached to the posterior coil of the sperm as a vesicle (*fig. 33*). The number of coils in the swimming sperm varies, but an average in material quickly killed is observed to be 2.75 coils. Numerous cilia grow from the whole of the surface of the band-shaped blepharoplast, their length sometimes surpassing more than twice the length of the body of the sperm.

OOGENESIS

The nuclei in the cells of the cushion region are generally larger than those of the rest of the prothallium. At first, successive mitoses that occur in the region to become the cushion divide cells anticleinally, so that there result a number of narrow parallel cells in a single row. In one of these cells, the next mitosis is pericleinal and gives rise to an archegonium initial (*fig. 34*). From the archegonium initial, by two successive mitoses, there are cut off a basal cell, a primary neck cell, and a central cell, or primary oogenous cell (*figs. 35, 36, 37*). Up to this differentiation of the central cell, the critical stage of each mitosis showed that the number of chromosomes is 64 or 66.

The central cell grows in size even after its nucleus begins to divide and becomes characteristically asymmetrical in form, due to the influence of the surrounding cells. The cytoplasm in the central cell consists of a fine fibrillar structure in general, which passes into an alveolar structure toward the periphery (*fig. 38*). There are observed in the central cell thread structures of various lengths, scattered throughout the whole cell, especially near the cell wall. Their staining capacity and general aspect recall similar structures which are observed in the central cell of the antheridium.

The resting nucleus in the central cell increases in size considerably (*fig. 38*). The nuclear reticulum consists of ragged chromatin material and three or more nucleoli are present. There now takes place a mitosis to divide the cell into neck-canal and ventral cells (*figs. 39-42*). The mitosis is typically vegetative; in the prophase the spirem is continuous and a single nucleolus is present, perhaps resulting from the union of the nucleoli in the resting stage. The appearance of longitudinal fissions in the chromosomes, their shortening and their arrangement in the equatorial plate, were exactly similar to those which were described in the vegetative mitoses of the prothallia. In the anaphase the two sets of daughter chromosomes show no difference in the amount of chromatin material (*figs. 41, 41a*), but after the telophase the two daughter nuclei become unequal in size (*fig. 42*). The inequality of these two sister nuclei has resulted neither from unequal distribution of the chromatin mass nor from the number of chromosomes, but is a matter of nucleo-cytoplasmic relation, i. e., on account of the position of the nucleus of the central cell, the cell

plate being laid down so as to divide the cytoplasm into two unequal cells. The immense amount of food material in the ventral cell causes its nucleus to grow more rapidly than that of the neck canal cell. At this time these two nuclei contain 66 or 64 chromosomes.

Then follows one more mitosis (simultaneous or successive) in each of these cells. The mitosis which divides the neck canal cell into two daughter cells is typical vegetative (*figs. 43-45*). In the telophase, when the daughter nuclei are formed, a cell plate is laid down as usual, but it soon begins to disorganize and finally disappears entirely. The direction of this mitosis is various, being sometimes parallel to the long axis of the archegonium, sometimes perpendicular to it, and sometimes oblique.

The ventral cell sooner or later divides into a ventral canal cell and an egg cell (*figs. 46-48*). No peculiarity is observed in this mitosis except in the metaphase (*fig. 46*), when there is almost always present a single dark staining body near the spindle, which possibly may be a persistent nucleolus, but its origin was not traced. The peculiar thread structure of unknown substance which is differentiated in the central cell is also continuously observed in the ventral cell, without any visible connection with the mitotic figure. In the telophase, the two daughter nuclei show a marked difference in size and shape, perhaps due to a nucleo-cytoplasmic relation, similar to the case previously observed (*fig. 47*). Several nucleoli appear in the young daughter nucleoli (*fig. 48*).

The characteristic curvature of the nucleus of the egg cell begins at the telophase of this division, and there is laid down by this time a dome-shaped cell plate which is of longer duration than that between the neck canal cells. The egg nucleus grows to an immense size and is very irregular in outline, whereas the neck canal cell and the ventral canal cell collapse and become mucilaginous, together with their disorganizing nuclei.

FERTILIZATION

The egg cell when ready for fertilization lies in the bottom of the archegonial cavity. Some of the material resulting from the disorganization of canal cells remains in the neck and ventral region of the archegonium as a slimy substance, even after the wide opening of the

neck admits free access of water. The dome-shaped cell wall which separated the egg cell from the ventral canal cell seems almost to disappear at the time of the disorganization of the ventral canal cell, so that the protoplast of the egg cell is exposed at the neck side to the dilute slimy substance, but this exposed surface retains its irregular dome-shape.

The cytoplasm of the egg cell has a fine fibrillar structure, very dense around the nucleus and vacuolate toward the periphery of the cell. The upper part of the nucleus becomes depressed, so that its concave side is directed toward the neck, and it presents a crescent form in the side view (*figs. 48, 49*). The chromatin material within the nucleus becomes transformed completely into a system of anastomosing and branching fine strands and small ragged clumps, characteristic of the resting condition. One, two, or more small nucleoli are present.

Several sperms enter the neck of the archegonium and reach the surface of the exposed protoplast of the egg cell at the so-called receptive spot (*fig. 50*). Normally one of these sperms penetrates the cytoplasm of the egg. The cytoplasmic layer which surrounds the egg nucleus at this time is rather shallow on the exposed surface in the direction of the neck, so that the sperm has to pass through only a shallow layer of the cytoplasm. When the sperm reaches the nucleus, the coil becomes shortened and the penetration of the sperm into the nucleus begins from the anterior region, followed gradually by the posterior region (*fig. 51*).

During the penetration of the sperm the chromatin reticulum of the egg nucleus persists as it was before, but a peculiar irregularity of the reticulum is observed to have occurred near the intruding sperm, which in penetrating has presumably caused a movement of the contents of the egg nucleus. After the sperm has completely penetrated into the egg nucleus, this disturbance of the homogenous arrangement of the chromatin reticulum is still observed so long as the sperm within the egg nucleus maintains its individual outline (*figs. 52a, b*).

It is also observed that there is present a clear region directly surrounding the body of the sperm within the egg nucleus, and this seems to be a transient stage preceding the disintegration of the body.

The disintegration takes place in the body of the sperm in the very situation it occupied within the egg nucleus. The chromatin material, which was aggregated into a compact and condensed structure up to this time, now begins to return into a loose anastomosed complex of numerous chromatin clumps and branched fine strands, similar to those which were observed during the metamorphosis of the nucleus to organize the sperm. Small new nucleoli make their appearance near or in the disintegrated region (*figs. 53a, b*). Whether these nucleoli result from the disintegrated blepharoplast or nuclear region of the sperm or from some different source was not determined. If the blepharoplast originated indirectly in the interior of the nucleus from nucleoli which might have escaped during the reconstruction of the nucleus of the spermatid mother cell in the last telophase (*fig. 11*), and by a temporary change of staining capacity may have escaped observation until they first become differentiated by stains as two bodies, it might be expected that it should now reappear or return again into nucleoli as it disintegrates. Thus close relationship between the blepharoplast and nucleolus might be established. However, this is entirely a speculative view, which lacks evidence sufficient to make the suggestion probable.

Disintegration of the body of the sperm proceeds still farther, the anastomosing chromatin material showing presently the ragged reticular structure characteristic of the resting condition (*figs. 54a, b*), and finally the chromatin material of the sperm nucleus becomes entirely anastomosed with that of the egg nucleus whose membrane now surrounds the chromatin of both egg and sperm (*fig. 55*).

The fusion nucleus in the resting condition contains two or more nucleoli, some of which have appeared at the time of disintegration of the sperm nucleus, while others have probably resulted from the union of nucleoli which already existed in the egg nucleus (*fig. 55*). Figures of the first segmentation division are obtained in material fixed a week after the entrance of the sperm had been observed. The spirem which is organized at the expense of the chromatin reticulum of the fusion nucleus has a continuous structure, without any indication of two chromatin constituents of maternal and paternal origin. Whether there exists one or two spirems was not determined, but even if there be present more than one spirem they are exactly similar in

thickness (*fig. 56*). Then the same process occurs as was described in vegetative mitosis. On account of the large number of chromosomes, the best stage for an accurate counting is obtained in the late anaphase, in which two sets of daughter chromosomes are regularly arranged near the poles (*fig. 57*). The polar view of this stage showed 128 or 132, or in rare cases 130, chromosomes (*fig. 58*).

Mitoses that occur following this first segmentation division, during the development of the embryo, were traced up to the appearance of the first leaf. The process is essentially similar, and 128 or 132 chromosomes are invariably present.

Discussion of cytological phenomena

The origin of the blepharoplast.—There have been many contributions concerning the origin and structure of the blepharoplast, not only in sperms of gymnosperms and pteridophytes, but also in zoospores of thallophytes.

In Cycas (IKENO 41, 42, 43, 48), Ginkgo (HIRASÉ 36, 37, 48), and Zamia (WEBBER 94, 95, 96), two blepharoplasts first are formed *de novo* in the cytoplasm of the body cell at some distance from the nucleus, and one of these blepharoplasts is included in each spermatid; accordingly the origin of the blepharoplast is cytoplasmic.

In Equisetum and Gymnogramme, BELAJEFF (6) observed the blepharoplasts as two deeply stained bodies on opposite sides of the nucleus previous to the final mitosis which differentiates the spermatid. In Marsilia SHAW (80) discovered two blepharoplastoids in the telophase of the mitosis which differentiates the grandmother cell of the spermatid. The blepharoplastoids disappear in the spermatid grandmother cell, in which two new blepharoplasts are formed at the poles of the spindle. BELAJEFF (7) examined the same form which SHAW had studied, and he found two centrosome-like bodies at the poles of the spindle of the mitosis which gives rise to the spermatid grandmother cell. These centrosome-like bodies are probably structures similar to the blepharoplastoids of SHAW; however, according to BELAJEFF's accounts, these structures do not pass into the cytoplasm to disappear, but accompany each daughter nucleus in the telophase and in the prophase of the next mitosis which occurs in the spermatid grandmother cell. This centrosome-like body divides and the two resulting

ones become situated at the poles of the spindle. The process is repeated until there is formed a spermatid in which this centrosome-like body remains and becomes the blepharoplast.

THOM's account (88) for *Adiantum* and *Aspidium* differs from those of the last two authors. In regard to the origin of the blepharoplast he states that the disappearance of nucleoli at the time of the appearance of the blepharoplast in the cytoplasm of the spermatid would suggest a relationship between them.

According to IKENO's account (45) of *Marchantia*, a centrosome is formed within the interior of the nucleus of the entire series of spermatogenous cells up to the spermatid grandmother cell. This body moves to the nuclear membrane and is thrust out into the cytoplasm. It then lies outside of the nucleus and becomes the functioning centrosome, dividing to form two centrosomes that separate and occupy the poles of the spindle. After the mitosis that gives rise to the spermatids, the centrosome remains to become the blepharoplast of the sperm.

Regarding the origin and structure of the blepharoplast of thallophytes, STRASBURGER (85, 87) expressed the following view, chiefly based on his study of the zoospores of *Vaucheria*, *Cladophora*, and *Oedogonium*. In all of these forms, he states, the blepharoplast arises from the outer plasma membrane (*Hautschicht*), the nucleus lying close to the plasma membrane at the time when the blepharoplast is formed. Another view, based upon the zoospores of *Hydrodictyon*, was advanced by TIMBERLAKE (89), who found that after nuclear multiplication had ceased, segmentation proceeded until uninucleate masses of protoplasm become separated from one another as zoospores. Then a blepharoplast was formed, lying in contact with the plasma membrane. But before the appearance of the blepharoplast, he adds, a granule may sometimes be observed close to the nucleus and it is possible that this is the first appearance of the blepharoplast. DAN-GEARD's study (21) on *Polytoma* also suggests some possible relationship between the blepharoplast and nucleus. In zoospores of this form, he finds that the blepharoplast is situated directly under the plasma membrane and that a delicate threadlike structure extends from it into the cytoplasm and sometimes ends at the side of the nucleus in a granule. DAVIS (24 a) traced the origin of the blepharo-

plast in *Derbesia*, in which the blepharoplast arises from the granules observed close to the nucleus. There is a time previous to the differentiation of the zoospore when the nucleus lies close to the cleavage furrow that finally separates the adjacent zoospore rudiments. Close to the nucleus he observed a number of granules which migrate toward the surface region of the protoplast of the zoospore and assume a ring-shaped arrangement. By the fusion of these granules the blepharoplast is established.

According to these results, excepting STRASBURGER'S on *Vaucheria*, *Cladophora*, and *Oedogonium*, the blepharoplast seems to originate in the interior of the cytoplasm (*Cycas*, *Ginkgo*, *Zamia*, *Equisetum*, *Marsilia*, *Gymnogramme*, *Onoclea*), sometimes from a position close to the nucleus (*Adiantum*, *Aspidium*, *Hydrodictyon*, *Polytoma*, *Derbesia*), and in still another case from the interior of the nucleus (*Marchantia*).

In *Nephrodium*, as has just been described, the mitoses which occur within the antheridium were all investigated, from the first spermatogenous cell until the final differentiation of the spermatid. The blepharoplasts were demonstrated by the differentiation of stains as two small deeply staining bodies appearing first within the cytoplasm of the spermatid mother cell. The nucleus of the spermatid mother cell is in the resting condition when these bodies appear, and during the mitosis which differentiates the spermatid the blepharoplasts are situated near the pole of the spindle. After the telophase one blepharoplast accompanies each daughter nucleus and there is established a spermatid with a single blepharoplast. Sometimes blepharoplasts appeared in spermatogenous cells of the four-celled stage of the antheridium, but it is certain that these four cells do not represent spermatogenous cells in an early stage previous to the formation of the spermatid mother cells; on the contrary, they are in this case real spermatid mother cells, the number of sperms produced in the antheridium then being only eight.

The relationship of the blepharoplast and the centrosome.—The centrosome in pteridophytes was figured first by HUMPHREY (11) and then by CALKINS (39) in the spore mother cells of such forms as *Osmunda*, *Psilotum*, *Adiantum*, and *Pteris*. One year after CALKINS' paper appeared, SHAW's account (80) of *Marsilia* and *Onoclea* was

published, according to which the blepharoplast has nothing to do with the mitosis as a center of the mechanism of nuclear division, but simply lies near the pole of the spindle. He does not regard the blepharoplast as a centrosome.

BELAJEFF'S conclusions (7) in reference to *Marsilia* oppose those of SHAW; for he believes that the blepharoplast always occupies the pole of the spindle and functions as a centrosome during mitosis. He claims that the blepharoplast in pteridophytes is homologous with the centrosome. He figures the division of the centrosome or blepharoplast in the spermatid mother cell previous to the division of the nucleus, and between the two separating daughter centrosomes or blepharoplasts a small central spindle is developed just as in certain animal cells.

IKENO (45) considers the blepharoplast of *Marchantia* to be actually a centrosome, as shown by its behavior during mitosis. He homologizes the *Nebenkörper*, the deeply staining body in the cytoplasm of the spermatid, with the blepharoplastoid of SHAW. Again he (46) discusses the homologous nature of the blepharoplast and centrosome in his paper entitled "*Blepharoplasten im Pflanzenreich.*" The *Nebenkern* of BELAJEFF, he suggests, is homologous with the deeply staining body (the *Körperchen*) in the spermatid of animals. Last year IKENO (47) reasserted his belief that the blepharoplast is a centrosome. He thinks that the bodies now called blepharoplasts may not all be homologous structures, but he holds that the blepharoplasts of pteridophytes, gymnosperms, liverworts, and myxomycetes are of centrosome origin either ontogenetically or phylogenetically.

The foregoing accounts of IKENO confirm BELAJEFF'S view regarding the homology of centrosome and blepharoplast. STRASBURGER (87) wrote at length on this subject seven years ago, and does not accept BELAJEFF'S view. He emphasizes the kinoplasmic character of the blepharoplast, whether it be a differentiated region of the plasma (as he believes for the zoospores of *Vaucheria*, *Cladophora*, and *Oedogonium*), or a special development in the interior of the cytoplasm (pteridophytes and gymnosperms). He thinks that all kinoplasmic structures, be they centrosomes, centrospheres, or blepharoplasts, hold a very close physiological relation to the structure of the nucleolus, so that the blepharoplast might occupy the position

of centrosome without being genetically connected with that structure.

In Nephrodium there is present no centrosome in the whole life-history, and the blepharoplasts which arise *de novo* in the cytoplasm of the spermatid mother cell do not play the part of a centrosome. Since centrosomes are not found in this form it is impossible of course to discuss any relationship between them. If any genetic relationship really exists between the two structures in the plant kingdom, evidence might be sought in some of the lower forms, such as Sphacelaria and some other species in Phaeophyceae, in which centrosomes are known and motile spores are produced.

Fusion of male and female nuclei.—The condition of sexual nuclei at the time of fertilization has been studied in numerous forms of different groups of plants.

Among thallophytes the fusion of gamete nuclei was observed in Closterium and Cosmarium (KLEBAHN 52), Rhopalodia (KLEBAHN 54), Cocconeis (KARSTEN 50), Sphaeroplea (KLEBAHN 55; GOLENKIN 30), Oedogonium (KLEBAHN 52), Vaucheria (OLTMANN 70; DAVIS 23), Coleochaete (OLTMANN 71), Fucus (FARMER and WILLIAMS 25, 26; STRASBURGER 86), Dictyota (WILLIAMS 97), Batrachospermum (SCHMIDLE 76; OSTERHOUT 72), Nemalion (WOLFE 98), Polysiphonia (YAMANOUCHI 100), Basidiobolus (FAIRCHILD 27), Albugo (WAGER 92; STEVENS 81, 82; DAVIS 22), Peronospora (WAGER 93), Pythium (MIYAKE 63; TROW 91), Achyla (TROW 91), Araiopora (KING 51), Sphaerotheca (HARPER 34), Pyronema (HARPER 35). No matter whether a condition of heterogamy is established or not in these forms after the union of gametes or gametangia, the male nucleus or nuclei pass into the cytoplasm of the egg or oogonium and union takes place between the male and female nuclei in the resting condition.

Similar conditions regarding the union of the sexual nuclei in angiosperms were described by STRASBURGER (84) for Monotropa, by SCHAFFNER (75) for Sagittaria, by COULTER (19, 20) for Lilium and Ranunculus, by MOTTIER (66, 67) for Lilium, by LAND (57) for Silphium, and by others. MOTTIER gives the most detailed account of chromatin at the time of the union, and figures the gamete nuclei as uniting with their chromatin in the resting condition. In the forms

mentioned above, the gamete nuclei, with a few exceptional cases, are of almost equal size at the time of union, and fusion of the chromatin of the two gamete nuclei takes place after the dissolution of the portions of the nuclear membranes which are in contact.

There are described among gymnosperms cases in which there is a marked difference in size between male and female nuclei, as *Thuja* (LAND 58), *Picea* (MIYAKE 64), *Abies* (MIYAKE 65), *Torreya* (ROBERTSON 73), *Sequoia* (LAWSON 59), *Cryptomeria* (LAWSON 60). In these species the sperm nucleus, being considerably smaller, becomes imbedded in a depression of the egg nucleus. However, the process of their union does not differ essentially from the cases observed in thallophytes and angiosperms, because the chromatin material of both gamete nuclei in resting condition fuses after the disappearance of the contiguous part of the nuclear membrane.

According to the accounts given by a number of authors there is still another case: in *Larix* (WOYCICKI 99) and *Taxodium* (COKER 18), the gamete nuclei which come into contact do not fuse, but the chromatin contents of both nuclei are kept in distinguishable maternal and paternal groups; while in *Pinus* (BLACKMAN 8; CHAMBERLAIN 17; FERGUSON 28) and *Tsuga* (MURRILL 68) the chromatin of sperm and egg nuclei remains separate, forming two spirems, and only after their segmentation into chromosomes are the two sets of structures brought together in the first cleavage spindle. In these cases there is never present a resting nucleus including both maternal and paternal chromatin within a common nuclear membrane.

In the case of *Nephrodium*, as already described, the sperm which entered into the egg nucleus was observed during a certain period without any visible change, entirely imbedded within the chromatin reticulum of the egg nucleus. The chromatin material which constituted the body of the sperm begins to disintegrate, and the final result is a reticular structure similar to that which we have noticed in the nucleus of the spermatid before the formation of the sperm. The reticular structures of both sperm and egg nuclei become anastomosed and mixed together entirely within the membrane of the egg nucleus. The spirem of the first segmentation division is organized from this reticulum as a continuous homogenous structure.

This process of disintegration of the body of the sperm within the

egg in *Nephrodium* accords with THOM'S and SHAW'S accounts. The presence of the resting nucleus including both maternal and paternal chromatin within a common nuclear membrane as described in *Nephrodium* was also described by IKENO (43, 44) in *Cycas* and *Ginkgo*, these two being the only forms among Cycadales and Ginkgoales in which fertilization has been traced to the complete union of the two gamete nuclei.

There is only one paper that gives details of fertilization in a bryophyte, a contribution by KRUCH (56) on *Riella*. In this form he states that after the sperm enters the egg, a male nucleus is organized which increases in size until it is about equal to the egg nucleus. This differs from the condition in *Nephrodium*, for the sperm does not enter into the egg nucleus, but two nuclei fuse side by side with their chromosomes fully organized.

With respect to the cytoplasmic structures of the sperm, all investigations among cycads and in *Ginkgo* agree that they are left behind in the cytoplasm of the egg before the gamete nuclei unite. A similar condition is reported in both *Adiantum* and *Aspidium*. In *Nephrodium* the author cannot confirm the foregoing view, since cases were observed in which the blepharoplast was not left behind in the cytoplasm. However, such cases do not of course necessarily mean that this structure takes part in what may be regarded as an essential part of the process of fertilization.

Conclusion

Since the present investigation on spermatogenesis, oogenesis, and fertilization, together with the preceding study on sporogenesis, has been made to trace out the complete history of the chromosome in the normal life-cycle of *Nephrodium* as preparatory to the study of apogamy, the general conclusions will be given in the subsequent paper. However, the two principal points established by the present investigation may be stated here: (1) the counting of the number of chromosomes is possible in the gametophyte; (2) the number of the chromosomes in the gametophyte is constant (64 or 66).

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EXPLANATION OF PLATES VI-VIII

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic objective 1.5^{mm} N. A. 1.30, combined with compensating ocular 18, except *figs.* 34-38, 41, 42, 46-50 drawn with compensating ocular 4, and *fig.* 33 drawn with compensating ocular 18 under higher magnification obtained by extending the tube. The plates are reduced to one-half the original size.

PLATE VI

Mitosis in the vegetative cells of the prothallium

FIG. 1. Prophase; nucleus with a continuous spirem.

FIG. 2. The nucleus after segmentation of spirem into a number of chromosomes, most of them before the stage of longitudinal splitting; two kinoplasmic caps with a few fibers are present.

FIG. 3. The nucleus after disappearance of membrane; chromosomes which are split longitudinally show a tendency to become arranged parallel to the axis passing between the two poles.

FIG. 4. Late prophase; daughter halves of chromosomes are in close contact with each other; they have shortened to L forms and are regularly arranged in an equatorial plate; the vertical arms of the L's are directed to the poles, while the horizontal arms are lying in the plate.

FIG. 5. Polar view of the stage shown in *fig. 4*; the horizontal arms of the L's are in regularly radiating arrangement and the optical section of the vertical arms appear like dots.

FIG. 6. Early anaphase; daughter chromosomes separating in a group.

FIG. 7. Late anaphase; two sets of daughter chromosomes near the pole.

FIG. 8. Early telophase; daughter chromosomes regularly arranged at poles.

FIG. 9. Polar view of the stage shown in the previous figure; 64 chromosomes present.

FIG. 10. Telophase; two daughter nuclei and a cell plate formed; arrangement of chromatin substance in daughter nuclei showing polarity.

Spermatogenesis

FIG. 11. Telophase preceding formation of spermatid mother cells; nuclei of spermatid mother cells just organized; two nucleoli present in one of the nuclei.

FIG. 12. Condition of nucleus later than stage shown in *fig. 11*; chromatin material very scanty in polar region, where nucleoli are present.

FIG. 13. Still later stage than that in *fig. 12*; arrangement of chromatin material still showing polarity.

FIG. 14. More advanced stage than that shown in *fig. 13*; nucleus of spermatid mother cell almost in resting stage; no polarity in distribution of chromatin material; two blepharoplasts within cytoplasm on opposite sides of nucleus.

FIG. 15. Nucleus of spermatid mother cell with spirem; two kinoplasmic caps with a few fibers; blepharoplast near pole of fibers.

FIG. 16. Spirem segmented into chromosomes; nuclear membrane begins to disappear at poles of nucleus; blepharoplast near the pole.

FIG. 17. Late prophase; nuclear membrane has completely disappeared; chromosomes split longitudinally; blepharoplast near center of spindle.

FIG. 18. Anaphase; two sets of daughter chromosomes on the way toward the poles.

FIG. 19. Early telophase; daughter chromosomes grouped at pole with regular arrangement; blepharoplast retains same position as before.

FIG. 20. Polar view of stage shown in *fig. 19*; 66 chromosomes evident.

FIG. 21. Late telophase; spermatid mother cell has divided into two spermatids, each containing a single blepharoplast near concave side of young nucleus; *Nebenkern* near each nucleus.

FIG. 22. One of the spermatids after the cellular connection has become loose and the regular arrangement has been lost; nucleus larger; chromatin reticulum composed of ragged clumps and two nucleoli present; blepharoplast applied very close to nuclear membrane; *Nebenkern* a little apart from the membrane.

FIGS. 23, 24. Two different views of same stage; *fig. 23* shows surface of blepharoplast; *fig. 24* the cross-section; *Nebenkern* moved near nucleus after blepharoplast has begun to grow and elongate.

FIGS. 25-27. Three different views of the same stage; *fig. 25* shows part of surface view; *fig. 26* longitudinal section; *fig. 27* cross-section.

FIG. 28. Nucleus of spermatid has become spiral; free end of blepharoplast wedge-shaped, the other end gradually tapering and along one edge a tapering end of the nucleus runs from the opposite direction and coalesces.

FIG. 29. Stage of spermatid nucleus later than that in *fig. 28*; coiled form of nucleus farther advanced; one end of nucleus, consisting chiefly of the blepharoplast, clearly visible; the other end, terminating in a sausage-shaped structure, slightly recognizable in the center below the *Nebenkern*, which is drawn lightly.

FIG. 30. Stage similar to that shown in *fig. 29*, viewed from different direction; end of nucleus terminating in sausage-shaped structure visible.

FIG. 31. Almost mature sperm within a spermatid; whole structure of 2.75 coils, of which 1.2 coils are blepharoplast, the rest the nucleus that terminates in a blunt end; long cilia borne on outer surface of blepharoplast; *Nebenkern* not drawn.

FIG. 32. Optical section of side view of stage shown in *fig. 31*; *Nebenkern* larger and near blunt end of sperm.

FIG. 33. Sperm fixed in free swimming condition; anterior end consists of blepharoplast which gradually covers only outer surface, and is replaced by band structure of nuclear substance which finally ends in a sausage form; *Nebenkern* present within vesicle.

PLATE VII

Oogenesis

FIG. 34. Cross-section of part of prothallium where cushion region arises later; nucleus in telophase of mitosis which forms archegonium initial.

FIG. 34a. Nucleus shown in previous figure under higher magnification.

FIG. 35. Nucleus of archegonium initial in metaphase of mitosis which cuts off basal cell.

FIG. 35a. Nucleus shown in *fig. 35* under higher magnification.

FIG. 36. Division into central cell and primary neck cell.

FIG. 37. Central cell or primary oogenous cell.

FIG. 38. By successive divisions of primary neck cell and growth of central cell, a characteristic archegonial projection is formed; cytoplasm of central cell with peculiar thread structures.

FIG. 39. Nucleus of central cell in early prophase; spirem continuous; a single nucleolus.

FIG. 40. Early metaphase; chromosomes, daughter halves in pairs, have shortened.

FIG. 41. Anaphase; cell growth continuing during mitosis.

FIG. 41*a*. Same mitotic figure shown in *fig. 41* under higher magnification; two sets of daughter chromosomes almost similar in amount; thread structures in cytoplasm.

FIG. 42. Telophase; two daughter nuclei unequal in size; ventral nucleus considerably larger than nucleus of neck canal cell.

FIG. 43. Prophase of mitosis in a neck canal cell.

FIG. 44. Anaphase of same.

FIG. 45. Telophase; a cell plate laid down between two neck canal cell nuclei; cytoplasm with vacuoles of various sizes.

FIG. 46. Section of archegonium after formation of two neck canal cells; the two nuclei in contact, the cell plate having already disappeared; nucleus in ventral cell in metaphase.

FIG. 46*a*. Nucleus of ventral canal cell shown in *fig. 46* under higher magnification; deeply staining body near spindle.

FIG. 47. Section of archegonium; ventral cell nucleus in telophase; two nuclei of neck canal cell in contact.

FIG. 47*a*. Two daughter nuclei shown in *fig. 47* under higher magnification; size and shape quite different; both contain a number of nucleoli; dome-shaped cell plate laid down separating egg and ventral canal cell.

FIG. 48. Section of archegonium after completion of egg, ventral canal cell, and neck canal cells; nucleus of egg larger than others.

FIG. 49. Section of archegonium with open neck; canal cells becoming mucilaginous, with disorganizing nuclei; egg nucleus irregular; single nucleolus present, due to union of several nucleoli seen when nucleus was organized.

PLATE VIII

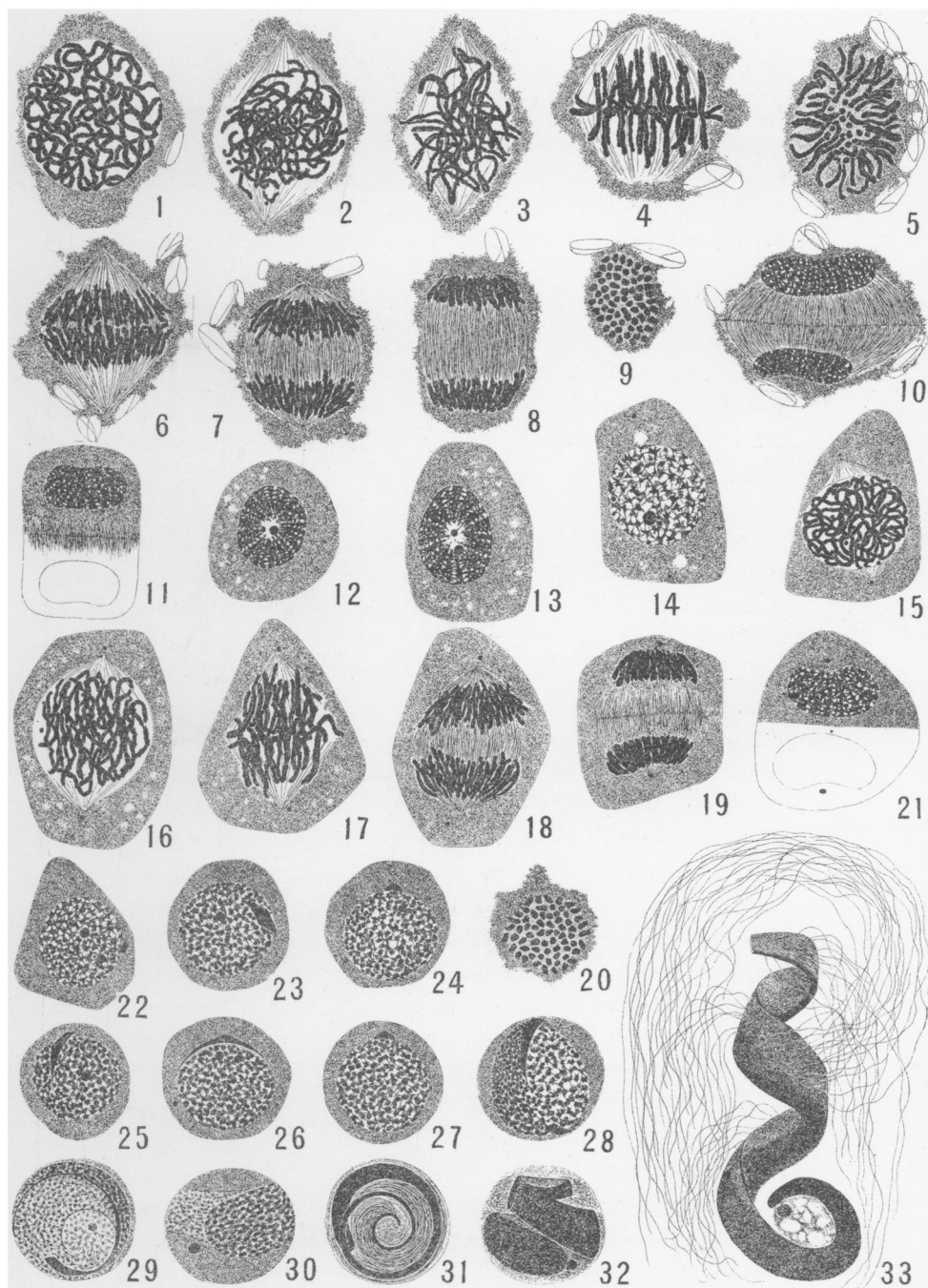
Fertilization

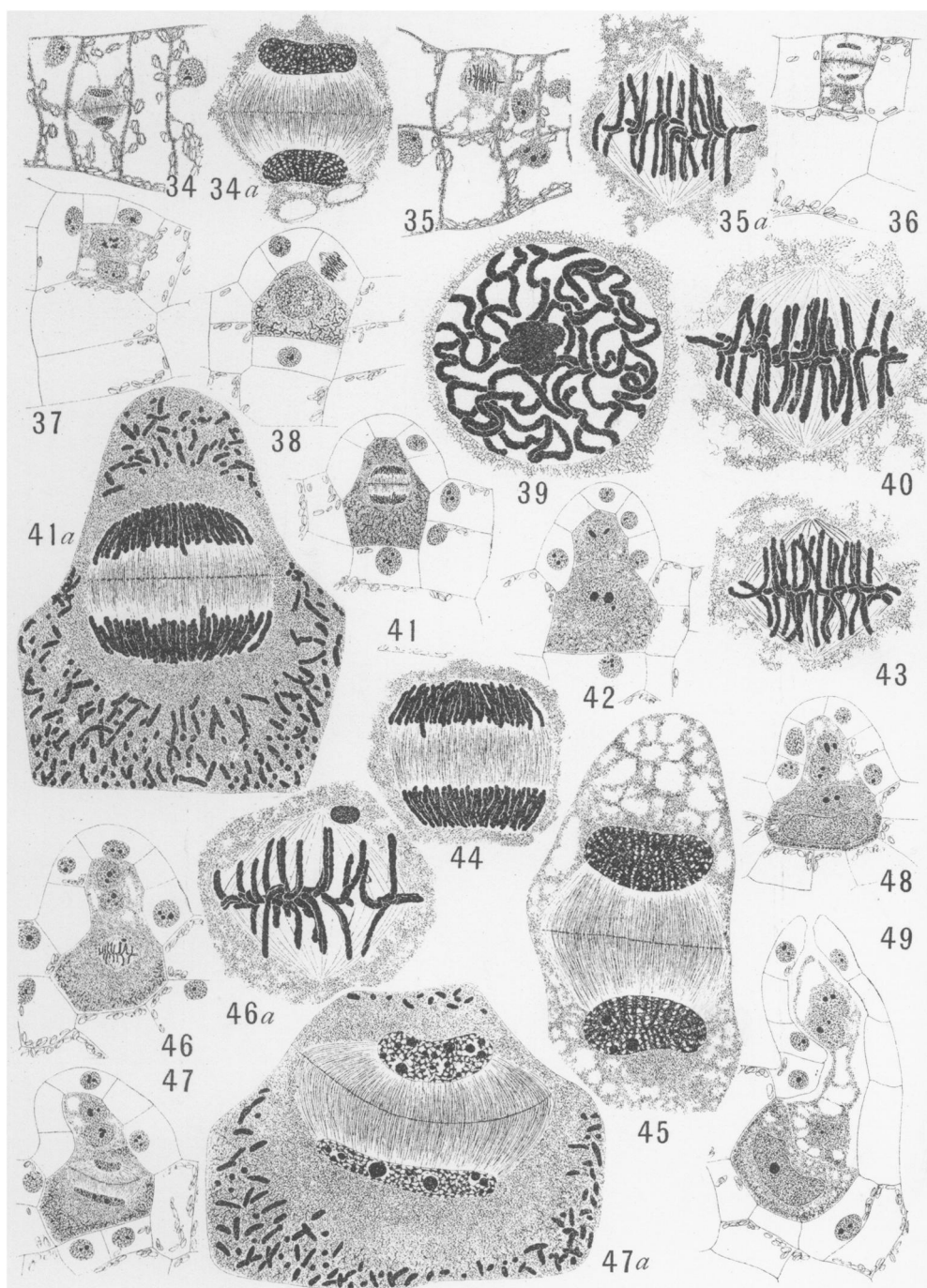
FIG. 50. Entrance of a number of sperms; many of them have already reached the egg.

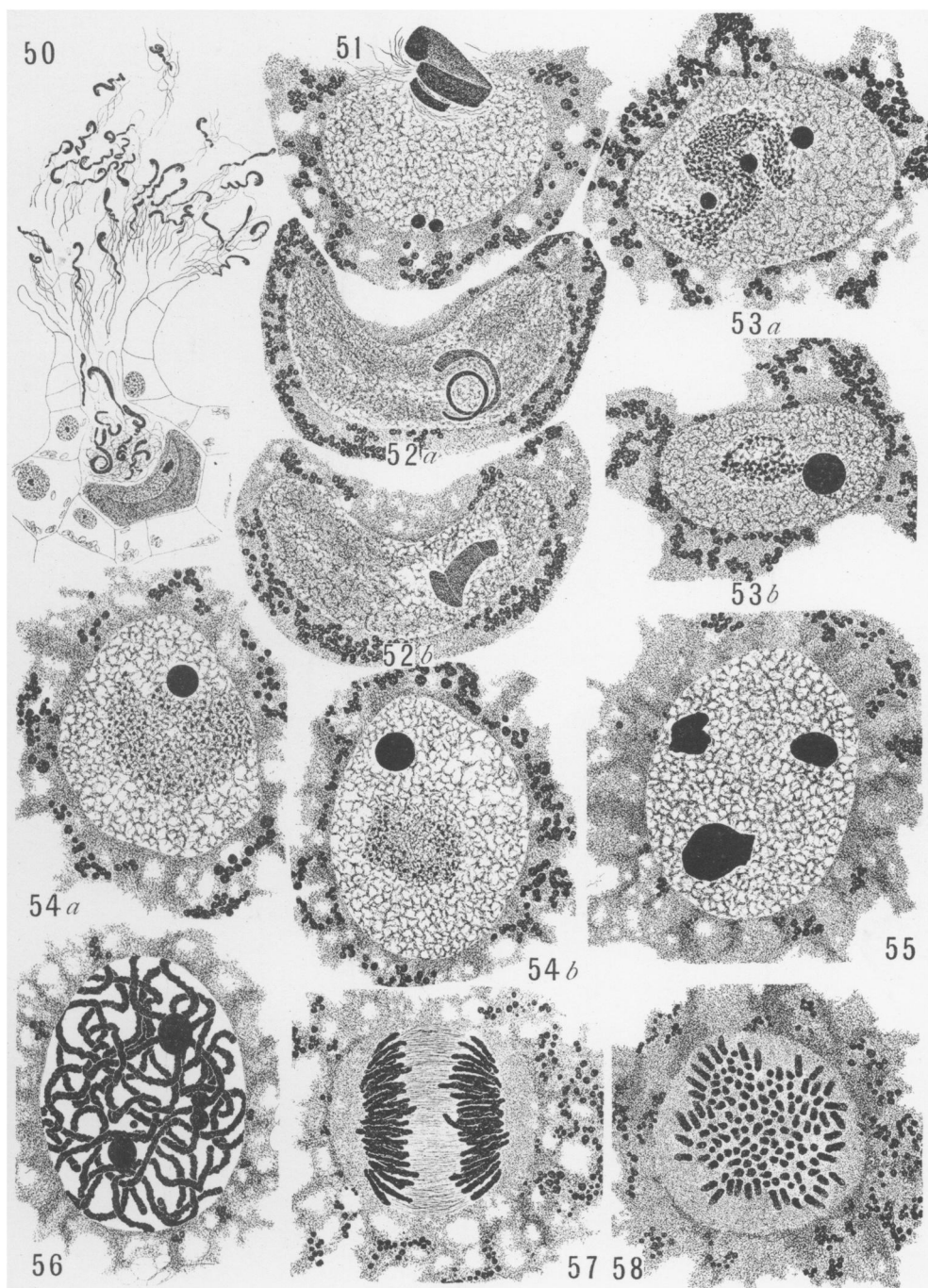
FIG. 51. Union of sperm and egg; sperm partly within egg nucleus; cytoplasm of egg enveloping nucleus very shallow toward neck.

FIGS. 52*a, b*. Two sections of same egg nucleus containing sperm, which has not changed; clear region surrounding sperm; upper side of figures toward neck.

FIGS. 53*a, b*. Two sections of same egg nucleus containing sperm in later stage than that shown in previous figures; disintegration begun; compact body







of sperm loosened into complex structure of chromatin clumps and fine strands; three nucleoli in region of disintegrating sperm; upper side of figures toward neck.

FIGS. 54*a, b*. Two sections of egg nucleus containing a disintegrating sperm in stage later than in last figures; neck toward the left.

FIG. 55. Fusion nucleus after complete union of the two chromatin reticulums of sperm and egg; ragged reticular structure homogeneous throughout; three nucleoli present; upper side of figure toward neck.

FIG. 56. Spirem of first segmentation division of fusion nucleus shows a continuous structure.

FIG. 57. Anaphase of same mitosis; the two sets of chromosomes regularly arranged at the poles.

FIG. 58. Polar view of stage shown in *fig. 57*; 128 chromosomes present.